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13. ABSTRACT ***(Maximum 200 Words)***

This is a two-year randomized trial of the effects of oral contraceptives on bone mass and stress fracture incidence among 150 female competitive distance runners in the age range 18-25 years. The Coordinating Center is at Stanford University and bone mass in being measured at five sites: Massachusetts General Hospital, University of California Los Angeles, University of Michigan, Stanford University/Palo Alto VA Medical Center, and Helen Hayes Hospital in West Haverstraw NY. Athletes are currently being recruited from the areas around these five clinical sites. In August 2001 we received IRB approval to expand our recruitment in the Los Angeles area to non-student runners, and this expansion has considerably increased our pool of eligible runners. To date, over all five clinical sites, 101 have been randomized and an additional 45 successfully screened and being scheduled for their bone densitometry. We expect to complete baseline examinations and randomization in early 2002. Final results will thus not be available until early 2004.

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(5) INTRODUCTION

Highly trained female athletes may experience loss of menses because of their participation in intense physical activity. Previous cross-sectional research has shown that women with exercise-induced menstrual irregularities have a significantly higher frequency of stress fractures and low bone mass than normally menstruating controls. Longitudinal studies suggest that these women are losing bone mass over time. Low serum estrogen levels are believed to be a principal cause of the bone loss. If so, re-establishing normal estrogen levels in these women should prevent or retard bone loss and decrease the incidence of stress fracture. This study is a two-year randomized trial of the effect of oral contraceptives on bone mass and stress fracture incidence among 150 female cross country runners in the age range 18-25 years. The Coordinating Center is at Stanford University and bone mass is being measured at five sites: the Massachusetts General Hospital, the University of California Los Angeles, the University of Michigan, Stanford University/Palo Alto VA Medical Center, and the Helen Hayes Hospital in West Haverstraw, NY. Athletes are being recruited from the areas around these five clinical sites.

(6) BODY

Below we summarize (a) the progress that was made through year 3, (b) the status of recruitment as of the end of year 3, and (c) our plans for completing the study.

<u>(a) Progress through year 3 (excluding recruitment, which will be described under [b] below):</u>

During the first year of the study the following accomplishments were reported: The study was introduced to coaches, athletes, student health services, and IRBs at many colleges, and procedures were implemented to work with these individuals and groups. Informational packets

were developed and sent to coaches, athletes, student health services, and others. Informed consent forms were developed and administered. Annual questionnaires, daily diaries, and six-month questionnaires were developed, pilot tested, and, in the case of the baseline questionnaire and daily diaries, used for data collection. Data entry programs were written and successfully used. A manual for the clinical sites was written and implemented. The Project Director (Kristin Cobb) spoke to athletes at many colleges and made the study known to athletes at high-profile races in the Stanford area. A randomization scheme was developed and implemented. Oral contraceptives were procured from Wyeth-Ayerst and procedures established for sending them to student health services and tracking them. Procedures were set up with the study's medical monitor.

During the second year of the study, athletes recruited during the first year were followed, additional runners were recruited, and, after lengthy negotiations, the Army and Helen Hayes Hospital IRBs agreed upon a consent form. Negotiations with the Army and UCLA IRBs regarding the UCLA informed consent form for non-collegiate runners were begun.

During the first year, recruitment was our biggest problem. After we revised our recruitment methods and expanded the scope of the study eligibility to include non-collegiate highly competitive runners in the age range 18-25 years, our main problem became the length of time it has taken to work out wording of the informed consent form that is mutually agreeable to the Army IRB and the IRBs at our clinical sites.

Most of the third year was particularly frustrating because the Army and UCLA IRBs did not reach agreement until August 2001 on the wording of a few components of the informed consent form for non-collegiate runners in the Los Angeles area. Finally, in August, after about one and a half years of negotiations, we received word from the Army that the consent form had

been approved. Since then we have established procedures for having the participants seen by a physician at UCLA and have been actively and successfully recruiting non-collegiate runners. Also during year 3, athletes recruited during the first two years have been followed and some additional runners have been recruited at the other sites. At this time last year, 82 athletes had attended their first clinical visit. Thus, by now, 82 athletes should have completed their first-year follow-up visits. In fact, 54 athletes have completed these visits. Of the other 28, 3 have withdrawn from the study, 8 only recently became due for their appointments and are in the process of scheduling, and 17 have been delayed in scheduling their appointments. Sixteen athletes have had both follow-up DXA measurements and have completed the entire study protocol.

One-year follow-up data from the first 35 participants have been analyzed, and a meeting has taken place with the study's medical monitor and statistician to review this analysis. They suggested that another meeting should be convened after follow-up data on 80 runners have been collected.

During year 3 we have also prepared a manuscript from the baseline data. The manuscript is appended and is about to be submitted for publication. The results are summarized below under Key Research Accomplishments.

(b) Recruitment through year 3: The focus of our recruitment efforts in the second and third year of the study shifted from collegiate to non-student athletes. At the end of the first year, 35 collegiate athletes had been randomized. During the second year, 14 additional collegiate athletes and 35 non-student athletes from the Stanford area were randomized for a total of 84. During the third year, an additional 17 non-student athletes were randomized from the Stanford

and New York areas, for a total of 101. An additional 45 athletes, mostly from the Los Angeles area, have recently been successfully screened and have scheduled or are in the process of scheduling their clinic appointments. This brings us to a total of 146 runners who have so far been randomized or who are scheduled to be examined and randomized.

Several recruitment procedures are being used in the Los Angeles area. We have recently mailed approximately 5400 flyers to women who have competed in running races within the past year. Coaches of local track clubs have been contacted and those clubs with women meeting our criteria were asked to pass on information to their runners via team websites, newsletters, and brochures. Flyers have been posted in retail sporting goods stores that tend to serve competitive runners. We are currently making arrangements to sponsor informational booths at local running races in Southern California, such as the Los Angeles County Race for the Cure 5K and the Long Beach Marathon. Through telephone calls and letters, we are also inviting local race participants who meet our qualifications to join the study. Finally, we recently visited six college and university cross-country teams to introduce the study and invite team members to participate. Other teams not visited because of time and travel constraints received informational packets.

(c) Plans for completing the study:

Now that we have received IRB approval to recruit non-collegiate runners in the Los Angeles area, we are confident that we will be able to enroll 150 runners who meet the study criteria, and that we will be able to do this by early 2002.

We will need to extend the period of funding for about two years beyond the originally planned project period of three and one half years. It still does not appear that we will need additional

money. As outlined in the Revised Annual Report submitted in February 2001, we have spent considerably less than anticipated over the first three years of the project for several reasons. We are only charged for bone densitometry when it is done, so we still have money for the remaining bone measurements. We had to reimburse travel expenses of collegiate athletes who were sometimes traveling over 100 miles, and reimbursement for the non-collegiate runners who are traveling much shorter distances is less. Recruitment was poor at our most expensive site for bone densitometry, the University of Michigan, while the Helen Hayes Hospital and Stanford, which were added after the study started, are less expensive. The Principal Investigator reduced her reimbursed time commitment to 5% in order to save money, and she is not using money from this project to travel to scientific meetings. The time of the Associate Project Director has been reduced and some of her tasks (e.g., data entry) are being done by less expensive Stanford undergraduates. With all these money-saving efforts, we should be able to complete the data collection without the need for additional funds, but we will need additional time. Finally, the Principal Investigator, Project Director, and Biostatistician are committed to completing the data analysis and writing up the results even if funding has run out before these tasks have been completed.

(7) KEY RESEARCH ACCOMPLISHMENTS: Attached is a manuscript describing some baseline results from the first 91 subjects. We found that (a) disorcered eating is strongly related to menstrual irregularity, (b) menstrual irregularity is associated with low bone mineral density independent of body weight and body composition, and (c) disordered eating is associated with low bone mineral density in the absence of menstrual irregularity.

(8) REPORTABLE OUTCOMES: None to date.

(9) CONCLUSIONS: We will have no firm conclusions to report until the end of the trial. However, in preparing interim data for our Medical Monitor, we can report that oral contraceptive use may be beneficial to spine and whole body bone mineral density in oligo/amenorrheic runners. Oral contraceptive use appears to bring about small gains in both lean and fat mass in young women runners.

(10) REFERENCES: None

(11) APPENDICES: Please see the attached manuscript.

TITLE: Disordered eating, menstrual irregularity, and bone mineral density in young female runners.

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ABSTRACT

Young female athletes are at risk for the female athlete triad, a syndrome composed of three interrelated disorders: disordered eating, menstrual irregularity, and low bone mineral density (BMD). The relationships between (a) disordered eating (elevated scores on an eating disorder inventory [EDI]) and menstrual irregularity (oligo/amenorrhea: 0-9 menses during the past year), (b) menstrual irregularity and low BMD, and (c) disordered eating and low BMD were investigated in 91 competitive female distance runners aged 18-26 years, 36% of whom were oligo/amenorrheic. An elevated score on the EDI (highest quartile) was associated with oligo/amenorrhea, after adjusting for percent body fat, age, miles run per week, age at menarche, and dietary fat, (OR [95% CI]: 4.6 [1.1-18.6]). Oligo/amenorrheic runners had lower BMD than eumenorrheic runners at the spine (-5%), hip (-5%), and whole body (-4%), even after accounting for weight, percent body fat, and EDI score. Eumenorrheic runners with elevated EDI scores had lower BMD than eumenorrheic runners with normal EDI scores at the spine (-11%), with trends at the hip (-5%), and whole body (-5%), after adjusting for differences in weight and percent body fat. Runners with both an elevated EDI score and oligo/amenorrhea had no further reduction in BMD than runners with only one of these risk factors, suggesting possible shared etiologic pathways between EDI score and oligo/amenorrhea. We conclude that, in young competitive female distance runners, (i) disordered eating is strongly related to menstrual irregularity, (ii) menstrual irregularity is associated with low BMD, and (iii) disordered eating is associated with low BMD in the absence of menstrual irregularity.

KEY WORDS: female athletes, long-distance, runners, bone mineral density, osteopenia, osteoporosis, amenorrhea, oligomenorrhea, eating attitudes, eating disorders, eating disorder inventory, female athlete triad

INTRODUCTION

The "female athlete triad"[36] is a combination of disordered eating, menstrual irregularity and osteoporosis/osteopenia seen in young female athletes. Disordered eating, which affects as many as 62% of young female athletes,[36] consists of restrictive eating behaviors that do not necessarily reach the level of a clinical eating disorder.[37] Women athletes with disordered eating may limit their caloric and/or fat intakes but maintain high training levels, often resulting in a state of chronic energy deficit. Among other adverse consequences, energy imbalance has been linked to depressed estrogen levels and amenorrhea or oligomenorrhea.[2-4,11,16,30,32,33] Amenorrheic/oligomenorrheic athletes on average have lower bone mineral density (BMD) than eumenorrheic controls.[1-18] This bone deficit may be related to an increased incidence of stress fractures[19-21] and may be only partially reversible,[22-24] putting women at risk for life-long health consequences.

The existence of the female athlete triad has been inferred from studies that established a relationship between eating behaviors and menstrual irregularity[2-4,11,16,30,32,33] and those that established a relationship between menstrual irregularity and low BMD.[1-18] Few studies have measured menstruation, diet, and BMD simultaneously, [4,11,16] and these were conducted before the female athlete triad was recognized as a distinct syndrome. Therefore, the female athlete triad has yet to be explored as a triad, and the complex relationships among all three components have yet to be established.

In this paper, we examine eating attitudes and patterns, menstrual status, and BMD in a group of 91 competitive female distance runners, using data collected at the baseline examination of a randomized controlled trial. We examine the etiology of menstrual irregularity in this population, specifically as it relates to diet and eating behaviors. We address the question of whether low body weight can explain the differences in BMD between eumenorrheic and oligo/amenorrheic athletes, as several researchers have suggested, [8-10,13] or if hypoestrogenism (assessed by oligo/amenorrhea) affects bone beyond the effects of low weight. Finally, we examine the relationship between disordered eating and BMD, a link that has not been well studied in female athletes.

MATERIALS/METHODS

We analyzed the baseline cross-sectional data from 91 competitive female long-distance runners, aged 18-25 years, who are participants in a randomized controlled trial to examine the effect of oral contraceptives on BMD in female runners.

Subjects

Women were recruited from inter-collegiate cross country teams, post-collegiate running clubs, and road race participants in the vicinity of Boston, Los Angeles, Palo Alto, CA, Ann Arbor, MI, and West Haverstraw, NY. Recruitment strategies included direct presentations to college teams, advertisements in print and on the internet, and mailed flyers. To be eligible, women had to run at least 40 miles/week during peak training times, and they had to compete in running races. Additionally, because the women were recruited as part of a randomized trial of oral contraceptives, they could not have used oral contraceptives or other hormonal contraception within 6 months prior to entering the study; they had to be willing to be randomized to take oral contraceptives or not to take them; and they could have no medical contraindications to oral contraceptive use. All women were required to visit a study physician or student health service staff member prior to enrollment in the study. Details of the study and testing procedures were explained to each subject, and a written, informed consent was obtained. The experimental protocol was approved by the Institutional Review Boards of Stanford University, the University of California, Los Angeles, the University of Michigan, the Helen Hayes Hospital, and Massachusetts General Hospital.

Questionnaire

A self-administered questionnaire was used to assess training regimen, injury history, and menstrual history. Women were asked to record the number of miles they ran per week during each competitive season (fall cross country, winter track, spring track) and the off-season (summer) in the past 12 months. From this information, average number of miles run per week was calculated in the past year. Questions were asked about the age when they started to run competitively, and about weight training and running surfaces (e.g, grass, dirt, pavement, etc).

Women reported the number of menses they had had in the past 12 months, and were classified, accordingly, as eumenorrheic (10 or more cycles in the past year), oligomenorrheic (4-9 menstrual cycles

per year)[49], or amenorrheic (fewer than 4 cycles in the past year).[49] Menstrual irregularity has been defined as 0-9 menses per year in previous studies of young women runners,[11,18,20-21] and we use this definition here. Both oligomenorrheic and amenorrheic athletes have previously been found to be hypoestrogenic[6,11,53] and to have reduced BMD.[6,8,11,17,53] In our study population, amenorrheic and oligomennorheic athletes were similar in BMD, EDI scores, and past menstrual irregularity, justifying their combination into a single group. Women recorded their age at menarche and indicated whether they had had 0, 1-3, 4-9, or 10-13 menses during each year after menarche. Total lifetime menses was calculated using the midpoint of each of these categories. The total number of past years of amenorrhea was calculated by summing the number of years for which women checked "0" or "1-3" periods, excluding the year of menarche and the current year. Past oligomenorrheic years was calculated similarly, except using the category "4-9" periods.

Diet and Eating Behaviors

An expanded version of the 97-item National Cancer Institute Health Habits and History food frequency questionnaire[46] was used to estimate usual nutrient intake during the prior 6 months. We modified the questionnaire to accommodate the special diets of college-aged female athletes by adding low-fat and non-fat versions of certain foods, vegetarian and vegan foods, ethnic foods, and sports nutrition products (such as Gatorade and Power Bars). The nutrient contents of the added foods were obtained from the U.S. Department of Agriculture Nutrient Database for Standard Reference, release 14 (54), and from food labels. Total intakes of energy, protein, fat, carbohydrates, calcium, phosphorous, iron, fiber, and vitamin C were calculated.

Three subscales (drive for thinness, bulimic tendencies, and body dissatisfaction) of the Eating Disorder Inventory[40] (EDI) were used to screen for subclinical eating disorders.[37] Responses on each EDI subscale were scored separately and also totaled.

Physical and bone measurements

At each of the five clinical assessment sites, height and weight were measured using standard stadiometers and balance-beam scales, respectively. Body mass index was calculated as kg/m^2.

Bone mineral density (BMD, g/cm^2) at the left proximal femur, spine, and whole body, and body composition (lean body mass and fat mass) were measured by dual energy x-ray absorptiometry (DXA; QDR 4500A, Hologic). The coefficient of variation for these machines is less than 1.0% for all sites (55). Each machine was cross-calibrated with a Hologic anthropomorphic spine phantom for participation in this study. Each site maintains a standard quality assurance program to determine whether adjustments or corrective actions are necessary for the machinery. All women were asked to refrain from heavy physical activity twenty-four hours prior to screening in order to minimize the effect of fluctuations in hydration status on body composition measurements.

Statistical Analyses

Statistical analyses were performed using the SAS statistical package, version 6.12 (SAS Institute, Cary, NC, U.S.A.). Means were compared between groups using t-tests for normally distributed variables and the Wilcoxon sign-rank test for non-normally distributed variables. Tukey's multiple comparisons test was used to compare mean BMD across more than two groups. Analysis of covariance was used to control for age, weight, and body composition.

The relationships between oligo/amenorrhea and certain training, diet, and physical characteristics were assessed by multiple logistic regression. Multiple linear regression was used to examine the effects of menstrual group and EDI score on BMD when considering EDI score as a continuous variable.

RESULTS

Thirty-six percent of the study sample met criteria for abnormal menses; 26% were oligomenorrheic and 10% were amenorrheic during the past year. Oligo/amenorrheic women were similar to the eumenorrheic women in age, weight, height, and body composition (**Table 1**). The oligo/amenorrheic women had menarche a mean of 1.2 years later and had had an average of 45% fewer menstrual periods in their lifetime than eumenorrheic women. They ran an average of 18% more miles per week than eumenorrheic women.

Disordered eating and menstrual irregularity

The women were divided into two groups (normal/elevated) by their total scores on three subscales of the eating disorder inventory (EDI). Women in the highest quartile of total EDI were classified as having elevated EDI scores compared to women in the lowest three quartiles. Women in the elevated EDI group had similar EDI values to those previously published for patients with anorexia nervosa[38] on the drive for thinness and body dissatisfaction subscales (**Table 2**). Athletes with elevated EDI scores reported 19% lower daily caloric intakes compared to women with normal EDI scores (**Table 2**), and reported that they obtained 25% fewer of those calories from fat. The groups were similar in consumption of other nutrients; the elevated EDI group had a somewhat lower calcium intake, but this was proportional to their lower caloric intake. Both groups, on average, consumed greater than 1200 mg of calcium per day, which is the U.S. recommended daily allowance (RDA) for this age group.

Of 23 women with elevated EDI scores, 65% had oligo/amenorrhea, whereas only 25% of 67 women with normal EDI scores did. Each of the three EDI subscales scores was higher in the oligo/amenorrheic group, but the drive for thinness EDI subscale had the strongest association with oligo/amenorrhea (**Table 3**). Oligo/amenorrheic athletes and eumenorrheic athletes were similar in daily nutrient profiles, except that oligo/amenorrheic athletes ate a lower percentage of their calories from fat (**Table 3**).

Table 4 shows odds ratios for several factors associated with oligo/amenorrhea. Being in the top quartile of EDI score conferred a four-fold increased odds of oligo/amenorrhea. Every one year of delayed

menarche was associated with a more than two-fold increase in the odds of oligo/amenorrhea. Odds of oligo/amenorrhea was also increased with greater miles run per week and was decreased with a higher percent body fat and with a higher percent fat intake, but the confidence intervals for these associations included one. Total caloric intake was not associated with menstrual disturbances.

EDI score and percent fat intake are modestly negatively correlated (Spearman rank correlation coefficient: r=-.34), and reduced fat intake may lie in the causal pathway between elevated EDI and oligo/amenorrhea. If dietary fat is removed from the logistic regression model, the OR for elevated EDI score increases from 4.6 to 6.7 (1.8, 25.6), suggesting that low fat intake accounts for some percentage of the association between elevated EDI and menstrual irregularity. EDI score was not correlated with miles run per week (Spearman rank correlation coefficient: r=.01), so increased training, though related to oligo/amenorrhea, does not mediate the relationship between elevated EDI scores and oligo/amenorrhea.

Menstrual irregularity and BMD

BMD was 5%, 5%, and 4% lower at the lumbar spine, total hip and whole body, respectively, in oligo/amenorrheic women compared to eumenorrheic women, after adjustment for weight, percent body fat, and EDI score **(Table 5)**. Adjusted and unadjusted BMD values were similar (**Table 5**); thus, although weight was strongly correlated with BMD at all skeletal sites (Pearson correlation coefficients: whole body: r=.43; hip: r=.40; and spine: r=.38), lower weight did not account for the association between menstrual irregularity and low BMD in this study population. Adjustment for age at menarche but did not affect any of the BMD values (data not shown).

Disordered eating and BMD

There were no differences in BMD between women with elevated EDI scores and women with normal EDI scores before adjusting for body size. However, women with elevated EDI scores were heavier (138.5 ± 3.2 lbs) and had a higher percent body fat (25.7 ± 1.1 %) than those with normal EDI scores (125.8 ± 1.7 lbs; 22.8 ± 0.6 %). Based on multiple linear regression, we would expect the women with elevated EDI to have .038 g/cm^2 greater BMD at the spine and hip and .028 g/cm^2 greater BMD at the

whole body due to their higher weight (correcting for their higher percent body fat). Once we adjusted for body weight and composition, women with elevated EDI scores had significantly lower BMD compared to women with normal EDI scores at the spine (-6%), with trends at the hip (-3%) and whole body (-4%).

Menstrual status modified the effect of EDI score on adjusted BMD (**Table 6**). Among eumenorrheic women, those with elevated EDI scores had significantly lower spine BMD and non-significant trends for lower hip and whole body BMD compared to women with normal EDI scores (**Table 6**). These differences were not attributable to past menstrual history, which was similar in the two groups. Among oligo/amenorrheic women, however, there were no trends for lower BMD among women with elevated EDI compared to women with normal EDI.

Multiple linear regression analysis confirmed the significant interactions between menstrual irregularity and total EDI score (0-69) on BMD at all skeletal sites (**Figure 1**). Among eumenorrheic runners, EDI score is inversely related to BMD. However, among oligo/amenorrheic women, BMD is not related to EDI score. Similarly, among women with low EDI scores, oligo/amenorrheic women had lower BMD than eumenorrheic women, but, among women with high EDI scores, menstrual irregularity was not related to BMD.

DISCUSSION

This study confirms the existence and significance of the "female athlete triad," a syndrome composed of three interrelated conditions: disordered eating, menstrual irregularity, and osteopenia/osteoporosis.[36] (i) We confirm that disordered eating in female runners is correlated with oligo/amenorrhea; (ii) we demonstrate that the association between oligo/amenorrhea and low BMD in female runners is independent of body weight and body composition; and (iii) we provide novel evidence that disordered eating is associated with low BMD in eumenorrheic women runners.

There was evidence of disordered eating in our study population. The women in our study who were in the highest quartile of total eating disorder inventory (EDI) score had similar values on 2 EDI sub-scales to patients with diagnosed anorexia nervosa;[38] they also had similar or slightly higher EDI scores than women athletes with established subclinical eating disorders.[37,40-42] The EDI measures only attitudes

about food and body size. However, we verified that elevated scores on the EDI translated to actual eating practices; women with elevated EDI scores reported lower total caloric intakes (by about 19% per day) and lower percent fat intakes (by about 25% per day) than women with normal EDI scores. None of the 91 women in our study indicated that she was dieting to lose weight (data not shown), suggesting that this observed dietary restriction represents long-term, chronic restriction, rather than temporary attempts to lose weight.

Women with elevated EDI scores had a four-fold increase in risk for oligo/amenorrhea, when controlling for other factors. Chronic energy deficit has previously been implicated in the etiology of athletic amenorrhea.[2-4, 26-27,28-35] Menstruation requires a small amount of energy, and halting menstruation may be an adaptive energy-conservation mechanism. In our study, the caloric restriction of the elevated EDI group did not explain their excess oligo/amenorrhea, nor did energy expenditure from training (which was not different between EDI groups). Rather, our data suggest that the development of oligo/amenorrhea in these women may have been mediated in part by a reduction of fat in the diet. Dietary fat, independent of total energy intake, has previously been shown to influence the menstrual cycle in non-athletic women;[43] however, this association has not previously been demonstrated in female athletes, and it will need to be verified in further studies. We speculate that women with disordered eating may have more aberrant patterns of eating, such as binging and fasting cycles; although total energy intake may not be altered, these patterns have potential to alter metabolic pathways, hormone levels, and, ultimately, menstruation.[50-52]

We found that delayed menarche was a strong predictor of later menstrual irregularity. Delayed menarche was correlated with menstrual irregularity in both women who initiated training prior to menarche and women who started training after menarche; thus, prior training does not explain the delay in menarche in the oligo/amenorrheic runners. This finding suggests that some women, such as those with a natural "runner's build," may be predisposed to menstrual irregularity, which would account for the existence of a subset of women with low total EDI scores (6.8 ± 1.8) and putatively sufficient caloric intake (2443 ± 210) who still lost their periods. Alternatively, disordered eating patterns may have developed pre-

menarche and pre-training in certain women which caused a delay in the onset of menarche and has subsequently continued to disrupt the menses. Our data were insufficient to evaluate this hypothesis.

We confirm numerous reports of reduced BMD in oligo/amenorrheic female athletes, in which the largest and most consistent effects have been demonstrated at the spine.[1-18] The differences in BMD between oligo/amenorrheic and eumenorrheic women that we observed were not attributable to differences in body weight, body composition, or EDI score. The magnitude of the difference was important; six percent of the oligo/amenorrheic young women had spine BMD values that would be considered osteoporotic, that is, a BMD value less than 2.5 SDs below young adult BMD[39] (<.772 g/cm^2 as measured with the Hologic densitometer). Forty-eight percent were osteopenic at the spine, a BMD between -1 SD and -2.5 SDs below the young adult value (.772-.937 g/cm^2). For comparison, none of the eumenorrheic athletes were classified as osteoporotic and only 26% were osteopenic at the spine.

Women with elevated EDI scores had low BMD for their weight. We attempted to determine if this reduction was due to oligo/amenorrhea or if disordered eating had an independent effect on bone. Eight women with high EDI scores were currently eumenorrheic and had no history of amenorrhea or delayed menarche. BMD was significantly lower at the spine and was lower at the hip and whole body in this subgroup compared to eumenorrheic women with normal EDI, after adjusting for weight, body composition, age, and age at menarche. Eumenorrheic women with elevated EDI were heavier and had more body fat than all other subgroups; they also started running at a later age (18.3 ± 1.3 yrs). Possibly, this group was resistant to loss of menses despite their aberrant eating because their menstrual cycles were established before they started running and/or because they were not as thin.[47] It is also possible that these women have subclinical menstrual abnormalities, such as anovulatory cycles and shortened luteal phase, which have been associated with spinal bone density losses.[45]

In our study population, having both disordered eating and oligo/amenorrhea was no more detrimental for bone than having either disorder alone. The numbers in some of our groups were small, however, and this observation should be verified in further studies. That there is no excess risk suggests that the two disorders share causal pathways. Both oligo/amenorrhea and disordered eating have been associated with a reduction of serum estrogen[32,33,35] which would be expected to have an adverse effect on

BMD. Accordingly, disordered eating may result in estrogen deficiency, which then may lead both to bone loss and menstrual irregularity.

Figure 2 summarizes risk factors for low BMD and menstrual irregularity, as well as possible pathways connecting elements of the female athlete triad. Disordered eating may decrease menstruation and BMD through estrogen deficiency and through alterations of other metabolic pathways. Low weight is an established independent risk factor for low BMD; in this study population, women weighing less than 115 pounds had a 5-fold increased odds of being osteopenic at any skeletal site (OR [95% CI]: 5.3 [1.6-17.0]). Some previous studies also found an association between low weight and oligo/amenorrhea,[2-3,8-9] though this study did not. Menstrual irregularity may be related to low BMD through mechanisms other than reduced estrogen.[45] Training factors and delayed menarche have direct influences on the menstrual cycle and on BMD.

It is difficult to explain why the athletes with elevated EDI scores were heavier than the women with low EDI scores even though they reported lower caloric and fat intakes. We would expect women with subclinical eating disorders to have lower weight and body fat, but this was not the case in our study. Possibly, heavier women are more prone to eating disorders because they are more dissatisfied with their natural body type. Alternatively, the EDI scale may identify women in the early stages of an eating disorder, but may miss women in the later stages, when they have already lost weight. We speculate that some of the women in the thinnest subgroup, the oligo/amenorrheic women with low EDI scores, may have had eating disorders, but may be in denial and/or may currently be satisfied with their bodies because they have succeeded in reaching a low weight. We further recognize that the division of the population into normal EDI/elevated EDI is simplistic. Disordered eating is a continuum and we have artificially imposed a division. However, multiple linear regression analysis, in which we treat EDI as a continuous variable, confirms our categorical data results.

A further limitation of our findings is that eating attitudes and body image perception may influence the reporting of food intake.[34] We cannot rule out the possibility that women with aberrant attitudes about body and food systematically underreport intake. As they are hyperconscious about their food intake, they may report what they think they should be eating rather than what they actually eat.

Food frequency questionnaires, despite other limitations, may actually reduce this tendency, as the total amounts of daily food, calories, and fat being reported are not readily quantifiable to the athlete.

In conclusion, we provide confirmation of the female athlete triad. We also conclude that the female athlete triad may be more hidden than previously realized. The women in this study were not excessively lean; the amenorrheic women averaged more than 22% body fat; the women with elevated EDI scores averaged more than 25% body fat. Thus, it may not be readily apparent to a coach or a physician that a woman is at risk for amenorrhea or is practicing disordered eating. However, both factors significantly affect bone, even in the absence of the other. Because there is a high prevalence of osteopenia in this population that may have serious life-long consequences, we recommend that all competitive women endurance athletes, particularly those in sanctioned collegiate programs, receive screening for eating disorders and menstrual irregularity and education about the female athlete triad.

Table 1. Mean ± one standard error of the mean for selected physical and reproductive characteristics, and training variables, by menstrual group.

Characteristic	Menstrual Group	
	eumenorrheic (*n=58*)	oligo/amenorrheic[†] (*n=33*)
Age (yrs)	21.7 ± .3	21.8 ± .5
Weight (lbs)	129.1 ± 1.9	128.1 ± 2.7
Height (inches)	65.1 ± .3	65.4 ± .5
BMI (kg/m^2)	21.5 ± .2	21.1 ± .3
Body fat (%)	23.9 ± .6	22.7 ± 1.0
Menses in past year (no. cycles)	11.5 ± .1	5.0 ± .5[*]
Menarche (age in years)	12.6 ± .2	13.8 ± .2[**]
Total lifetime menstrual periods (no. cycles)	89.5 ± 4.4	49.5 ± 4.0[**]
Started running (age in years)	14.5 ± .5	14.7 ± .7
Amount of running (miles/wk in past 12 months)	33.0 ± 1.2	39.0 ± 2.2[‡]

[†]Oligo/amenorrhea was defined as 0-9 menses over the past 12 months.
[*]$p<.0001$, Wilcoxon sign-rank test.
[**]$p<.0001$, t-test.
[‡]$p<.05$, t-test.

Table 2. Mean ± one standard error of the mean for selected diet and nutrition characteristics by eating disorder inventory (EDI) group and, for comparison, a previously published anorectic group.

Characteristic	EDI Group (this study)		anorectics (previously published)[†]
	normal EDI (*n=67*)	elevated EDI[*] (*n=23*)	(*n=155*)
EDI scores[*]			
Drive for thinness subscale (0-21)	1.6 ± 0.3	16.3 ± 0.8[**]	13.8 ± 0.5
Bulimia subscale (0-21)	0.8 ± 0.2	3.2 ± 0.7[**]	8.1 ± 0.5
Body dissatisfaction subscale (0-27)	3.6 ± 0.5	16.0 ± 1.2[**]	15.5 ± 0.6
total (0-69)	6.0 ± 0.8	35.6 ± 1.8[**]	37.4 ± 0.9
Daily Nutrient Intake			
Calories (kcal/d)	2346 ± 112	1904 ± 148[‡]	
Fat (% of total calories)	18.7 ± .8	14.0 ± 1.0[£]	
Protein (% of total calories)	16.4 ± .3	16.0 ± .8	
Calcium, mg	1467 ± 96	1300 ± 147	
Fiber, g	30.6 ± 2.5	26.4 ± 2.2	
Vitamin C, mg	291 ± 23	247 ± 23	
Iron, mg	23.6 ± 2.3	20.0 ± 1.9	

[*]EDI score is the total score from three subscales of the Eating Disorder Inventory (EDI), Garner and Olmstead.[38] Elevated scores are defined as the highest quartile (≥23). One subject is missing EDI scores; therefore she is removed from all analyses involving EDI.
[†]Average scores for anorexia nervosa patients as published by Garner and Olmstead.[38]
[**] elevated EDI group vs. normal EDI group, p<.0001, Wilcoxon sign-rank test.
[‡] elevated EDI group vs. normal EDI group, p<.05, t-test.
[£] elevated EDI group vs. normal EDI group, p<.01, t-test.

Table 3. Mean ± one standard error of the mean for selected diet and nutrition characteristics by menstrual group.

	Menstrual Group	
Characteristic	eumenorrheic (*n=58*)	oligo/amenorrheic (*n=33*)
EDI scores*		
Drive for thinness subscale (0-21)	3.3 ± 0.7	9.3 ± 1.4§
Bulimia subscale (0-21)	0.9 ± 0.2	2.3 ± 0.5**
Body dissatisfaction subscale (0-27)	5.4 ± 0.8	9.3 ± 1.5**
total (0-69)	9.6 ± 1.5	20.9 ± 3.0§
Daily Nutrient Intake		
Calories (kcal/d)	2241 ± 121	2219 ± 147
Fat (% of total calories)	18.7 ± .9	15.3 ± 1.0¥
Protein (% of total calories)	16.3 ± .4	16.3 ± .5
Calcium, mg	1418 ± 106	1437 ± 123
Fiber, g	28.1 ± 2.2	32.0 ± 3.7
Vitamin C, mg	283 ± 23	274 ± 28
Iron, mg	22.2 ± 2.6	23.6 ± 2.1

*EDI score is the total score from three subscales of the Eating Disorder Inventory (EDI), Garner and Olmstead.[38]

§oligo/amenorrheic vs. eumenorrheic, p<.005, Wilcoxon sign-rank test

**oligo/amenorrheic vs. eumenorrheic, p<.05, Wilcoxon sign-rank test

¥eumenorrheic vs. oligo/amenorrheic, p<.05, ttest

Table 4. Odds ratios (and 95% confidence intervals) for the association between selected characteristics and oligomenorrhea/amenenorrhea.*

Characteristic	Odds Ratios (95% CI)
Elevated EDI score (≥ 23 vs <23)	4.56 (1.12, 18.61)
Menarche (each 1 year later)	2.45 (1.46, 4.11)
Miles/wk (every 10 miles)	1.64 (0.96, 2.79)
Dietary fat (every 5% of total calories)	.61 (0.36, 1.03)
Body fat (every 5%)	.56 (0.30, 1.07)

*Adjusted for age and each of the other variables in the table by multiple logistic regression.

Table 5. Observed and adjusted* spine, hip, and whole body bone mineral density (BMD, g/cm^2 ± one standard error of the mean), by menstrual group.

	Menstrual Group	
	eumenorrheic (*n=58*)	oligo/amenorrheic† (*n=33*)
spine BMD		
observed	1.01 ± .013	.93 ± .018**
adjusted*	1.00 ± .013	.95 ± .018‡
total hip BMD		
observed	1.00 ± .015	.95 ± .020‡
adjusted*	1.00 ± .014	.95 ± .019‡
whole body BMD		
observed	1.12 ± .011	1.08 ± .015‡
adjusted*	1.12 ± .010	1.08 ± .014‡

*Adjusted for age, body weight, percent body fat, and EDI score by analysis of covariance.
† Oligo/amenorrhea was defined as 0-9 menses over the past 12 months.
**eumenorrheic vs. oligo/amenorrheic, $p < .005$, t-test.
‡eumenorrheic vs. oligo/amenorrheic, $p < .05$, t-test.

Table 5. Observed and adjusted* spine, hip, and whole body bone mineral density (BMD, g/cm^2 ± one standard error of the mean), by menstrual group.

	Menstrual Group	
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total hip BMD		
observed	1.00 ± .015	.95 ± .020‡
adjusted*	1.00 ± .014	.95 ± .019‡
whole body BMD		
observed	1.12 ± .011	1.08 ± .015‡
adjusted*	1.12 ± .010	1.08 ± .014‡

*Adjusted for age, body weight, percent body fat, and EDI score by analysis of covariance.
† Oligo/amenorrhea was defined as 0-9 menses over the past 12 months.
**eumenorrheic vs. oligo/amenorrheic, $p < .005$, t-test.
‡eumenorrheic vs. oligo/amenorrheic, $p < .05$, t-test.

Table 6. Observed and adjusted* spine, hip, and whole body bone mineral density ($g/cm^2 \pm$ one standard error of the mean) by combined menstrual and eating disorder inventory (EDI) groups.

	Group			
	1	2	3	4
EDI score group‡	normal	normal	elevated	elevated
Menstruation	eumenorrhea	oligo/amenorrhea†	eumenorrhea	oligo/amenorrhea
N	50	17	8	15
Mean weight (pounds ± SE)	126.3 ± 1.8	123.5 ± 4.3	146.4 ± 5.7	133.4 ± 3.2
spine BMD ($g/cm^2 \pm$ SE) *				
observed	1.02 ± .015	.90 ± .024[a]	.97 ± .027	.97 ± .025
adjusted*	1.02 ± .014	.93 ± .024[a]	.91 ± .036[b]	.96 ± .025[c]
total hip BMD ($g/cm^2 \pm$ SE) *				
observed	1.00 ± .016	.91 ± .038[b]	1.00 ± .023	.98 ± .032
adjusted*	1.01 ± .015	.93 ± .027[b]	.96 ± .040	.96 ± .027
whole body BMD ($g/cm^2 \pm$ SE) *				
observed	1.12 ± .013	1.07 ± .018	1.12 ± .029	1.09 ± .016
adjusted*	1.13 ± .010	1.08 ± .019	1.07 ± .028	1.08 ± .020

‡EDI score is the total score from three subscales of the Eating Disorder Inventory (EDI), Garner and Olmstead.[38] Elevated scores are defined as the highest quartile (≥23). One subject is missing EDI score; therefore she is removed from all analyses involving EDI.

† Oligo/amenorrhea was defined as 0-9 menses over the past 12 months.

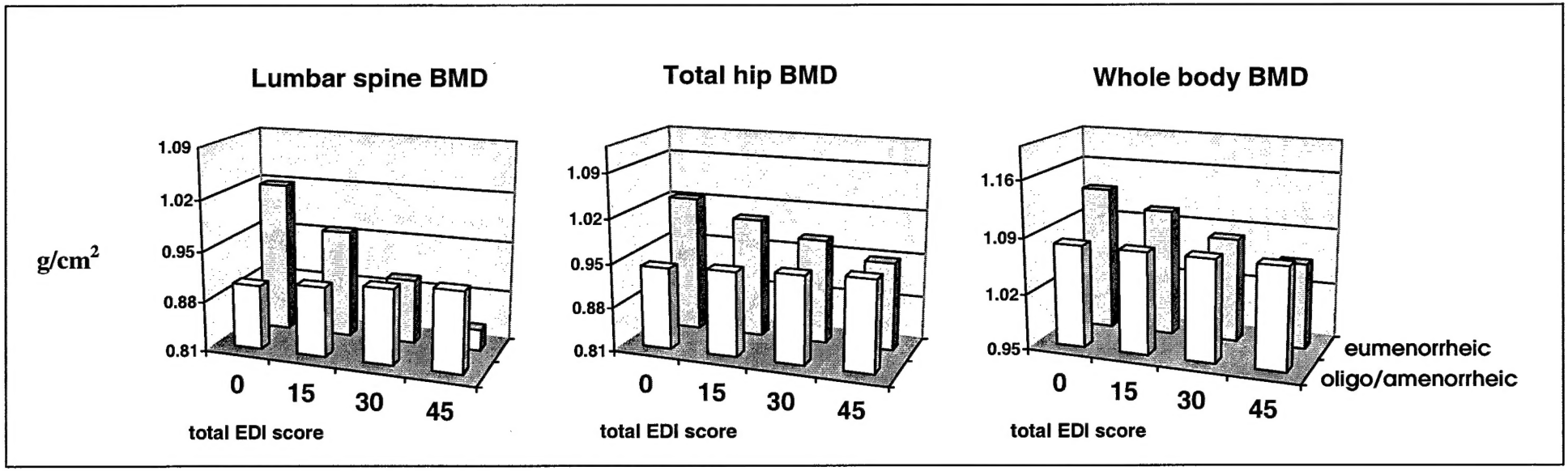
*Adjusted for body weight, percent body fat, age, and age at menarche by analysis of covariance.

[a]Group1 vs. Group 2, $p<.005$, Tukey's test for comparing multiple group means.

[b]Group1 vs. Group 2; Group1 vs. Group 3, $p<.05$, Tukey's.

[c]Group1 vs. Group 4, $p<.10$, Tukey's.

Figure 1. Mean BMD (g/cm^2) at the spine, hip and whole body by menstrual status and varying levels of total EDI score (from multiple linear regression**).



**The means are based on the following multiple linear regression results (adjusted for age, weight, and percent body fat):

Regression coefficients (standard error)		
menstrual status†	EDI score (0-69)	interaction: menstrual status x EDI score
-.12 (.033)	-.004 (.001)	+.0043 (.002)
-.089 (.036)	-.002 (.001)	+.002 (.002)
-.060 (.025)	-.002 (.001)	+.002 (.001)

† Menstrual status equals 1 if the woman is oligo/amenorrheic and 0 if the woman is eumenorrheic.

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